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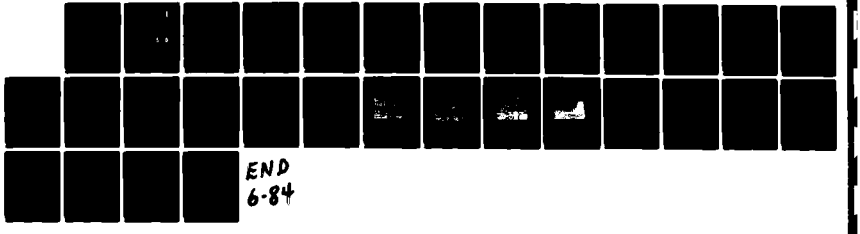

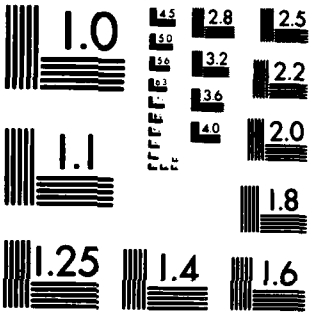

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BIOLOGICAL APPLICATIONS AND EFFECTS OF OPTICAL MASERS

PROGRESS REPORT

William T. Ham, Jr., Harold A. Mueller, John J. Ruffolo, Jr.,
Stephen F. Cleary, A.M. Clarke, R. Kennon Guerry, DuPont Guerry III

August 1981

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1. Abstract

A MILES prototype GaAs laser has been installed in the visual acuity room where a trained monkey will be exposed for 1000 s on a 5 day basis during his visual acuity testing program. Although previous exposures of the rhesus retina to 2 GaAs lasers in the pulsed mode did not produce funduscopically visible effects, a third GaAs laser (830 nm) modulated at 20.4 MHz did produce funduscopically visible damage at power levels too low to cause thermal damage to the retina. Possible explanations are discussed. Histological examination was not successful in locating the lesions. Further LM and EM analysis will be pursued. An investigation of near IR thresholds for cw radiation provided by the 2500 W xenon optical source has been completed for short bandpass wavelengths peaked at 820, 860 and 910 nm. All lesions were judged to be thermal in nature. The action spectrum for near UV radiation minimal retinal lesions has been completed on 3 aphakic monkeys. Radiant exposures needed to produce a minimal lesion range from 15 $J.cm^{-2}$ at 405 nm to 5 $J.cm^{-2}$ at 325 nm for 100 s exposure durations and from 23 to 5.1 $J.cm^{-2}$ for 1000 s. The rhesus retina is about 6 times more sensitive to near UV radiation as compared to blue light. Both funduscopic and histological observations indicate that the UV lesion differs from a blue light lesion in important respects. Notably, the photoreceptors, both rods and cones, are destroyed by near UV radiation. Both rhodopsin and the cone pigments have high absorption peaks in the UVA. The long term repetitive exposure program to UV and short wavelength radiation continues, but the trained animal which started exposures to the waveband 330-490 nm on October 10, 1979, died on June 5, 1981 after an accumulated exposure to the retina and lens of 8069 and 1556 $J.cm^{-2}$ respectively. Subtle retinal damage was funduscopically observable but no changes were noted biomicroscopically between the exposed and unexposed lenses. The other trained animal began exposures to 330-420 nm on February 20, 1980 and continues to receive daily exposures of 1000 s for 5 days per week. As of June 5, 1981, the accumulated radiant exposure to the lens was 1148 $J.cm^{-2}$. No changes have been noted between the exposed and the control lenses. Fluorescence and transmissivity measurements are in progress to detect early lens changes. An aphakic animal is undergoing training for long term repetitive exposures to the waveband 330-420 nm. The rhesus retina is so sensitive to near UV radiation that irradiances at the cornea must be restricted to 100-200 $\mu W.cm^{-2}$ according to present calculations. A new acoustically modulated krypton-argon laser has been installed for an investigation of retinal sensitivity to damage as a function

of wavelength, pulse repetition frequency and pulse duration. Preliminary data at the highest modulation frequency which the system can deliver (20.4 MHz, 49 ns pulses) for wavelengths 514.5, 488 and 458 nm does not show any significant difference from our previous cw data on 8 laser lines.

2. Investigation of Ocular Hazards of GaAs Radiation.

a. Repetitive Long Term Exposure

The MILES prototype GaAs laser arrived in April and has been installed in the sound-proof viewing room used for visual acuity tests. A "hot" mirror (transmits wavelengths below 700 nm and reflects the IR) is placed across the viewing screen at the proper angle to reflect the GaAs beam into the monkey eye. Spectroscopic analysis has shown that there is no change in spectral distribution of the light transmitted through the mirror. Tests both on ourselves and the trained monkey have shown that the "hot" mirror does not affect viewing conditions. Power at the monkey eye is approximately 860 μW of near infrared radiation. The diameter of the beam is about 1 cm to the $1/e^2$ points of the Gaussian distribution at the monkey eye. The bandwidth is about 30 nm peaking at 910 nm. A baseline of animal performance under the new conditions is in progress. When this is completed a daily radiation schedule of 1000 s of exposure per day for 5 days per week will be established.

b. Exposure of Anesthetized Monkeys to CW and Pulsed GaAs Radiation

Previous exposures of the rhesus retina to two GaAs lasers operating in the cw mode and in the pulsed mode at 1600 or 1700 Hz and wavelengths 830 and 904 nm did not produce any funduscopically visible effects, even after radiant exposures up to 7800 $J.cm^{-2}$. These experiments were reported in the previous research progress report dated 1 Sept. 1978 - 31 Aug. 1980.

More recently we have exposed one rhesus eye to another GaAs laser operating at 830 nm with a PRF of 20.4 MHz. A Galilean telescope reduced the divergence of the beam to 6.44 milliradians so that the entire beam could be directed on the retina of the dilated, anesthetized animal. The calculated spot size on the retina was 87 μm in diameter based on the emmetropic focal length of the rhesus eye (13.5 mm). Power level at the cornea was 330 μW as measured with a Scientech calorimeter. Taking 0.83 as the transmittance of the OM for 830 nm, the calculated retinal irradiance to the 1/e points of the Gaussian distribution was 4.6 $W.cm^{-2}$. Four exposures of 1000 s each (radiant exposure 4600 $J.cm^{-2}$) produced 4 lesions which were funduscopically visible immediately postexposure, one in the foveola, two in the

fovea and one in the parafovea. Unfortunately, this animal was not sacrificed for analysis by LM and EM until 4 weeks postexposure, which may explain why no traces of the lesions could be located histologically. Future plans call for sacrifice immediately postexposure so that histological and ultrastructural data can be obtained.

The above findings were startling for a number of reasons. A simple calculation using Mainster's mathematical model showed that the maximum temperature in the retina during exposure was below $1^{o}C$, making it very unlikely that thermal effects were involved. All previous exposures to GaAs lasers, both cw and pulsed, had given completely negative results. The major difference between this laser and those which had been used in the past was the PRF, 20.4 MHz as opposed to 1600 or 1700 Hz, yet peak power was much higher in the latter two lasers. There is a possible explanation in terms of David Sliney's memorandum, "Ultra-short pulse behavior of laser diodes - safety implications," dated 27 Jan. 1981. Solid state semiconductor laser diodes have an inherent tendency to become relaxation oscillators at a very high frequency which produces light pulses less than a nanosecond in duration. It is just possible that our 20.4 MHz laser diode was producing such light pulses, whereas neither the 1600 or 1700 Hz laser diodes went into oscillation. There is also evidence that these extremely short pulses produce frequency doubling which would expose the retina to 415 nm light. However, the power level is too low to produce a blue light lesion. A more likely hypothesis is if 100 ps pulses are generated by high frequency oscillations that they might set up sonic transients in the retina similar to those we noted at Los Alamos for 30 ps pulses of 1064 nm radiation. In those experiments we found that single pulses of 8 to 16 μJ produced a minimal lesion which was funduscopically visible immediately after exposure. To quote Sliney's memorandum, "It is premature to make firm recommendations regarding the increased hazards of such diodes. At present we recommend that needless staring into such laser sources be strongly avoided even if the source is presumably safe by present criteria."

We plan further experiments with this laser diode modulated at 20.4 MHz. Especially, we shall try to get some histological and ultrastructural data on the nature of the lesion.

c. Retinal Exposure to Short Bandpass Wavelengths Peaked at 820, 860 and 910 nm as Produced by the 2500 W Xenon Lamp

We have completed an investigation of near IR thresholds for cw radiation provided by the 2500 W xenon optical source with quartz optics. Exposure times range from 10 s to 1000 s. It was possible to get sufficient power through 10 nm half bandwidth filters at 820 and 860 nm. However, at 910 nm it was necessary to use a 55 nm bandpass. At each wavelength we determined the shortest exposure for the available power which would produce a minimal lesion in the rhesus retina. All animals were anesthetized and dilated so that the entire beam entered the eye with a divergence which produced a spot size of 500 μm on the retina. Results for the three wavelengths are given below in Tables I, II, and III. Minimal lesions were funduscopically visible within 24 hours after exposure and were smaller than the spot size on the retina. All lesions were judged to be thermal in nature.

3. The Action Spectrum for Near UV Radiation in the Aphakic Monkey.

The hypothesis that retinal sensitivty to photic damage would continue to increase as the wavelength decreased into the near UV region of the spectrum has been substantiated in the rhesus monkey. Three aphakic eyes (animals operated on by Dr. DuPont Guerry, III) were exposed to the wavelengths 405, 380, 350 and 325 nm as provided by the 2500 W xenon source with quartz optics and 10 nm bandwidth interference filters. These filters were blocked to 0.01% for all other wavelengths from X-rays to 3.5 μm except the designed 10 nm waveband.

Animals were anesthetized and dilated so that the entire radiation beam entered the eye with a divergence set to produce a 500 μm spot size on the retina. The power P_c at the cornea was measured with an Eppley black body receiver which had been checked against a Scientech calorimeter at each wavelength. The power level required to produce a minimal lesion as observed funduscopically immediately post-exposure was determined for 100 and 1000 s exposures in each aphakic eye for all four wavelengths. Usually it required 5 to 7 exposures to define a minimal lesion at each wavelength in each eye. All exposures were paramacular.

In Figure 1, Maher's data for the rhesus monkey (1) has been used to plot transmittance vs wavelength in nm through the cornea, aqueous and vitreous with lenticular transmittance excluded to approximate the aphakic eye. The area between the c-curve

TABLE I

Corneal power, retinal irradiance and radiant exposure for cw radiation

820 nm (10 nm half bandwidth)

Exposure Time (sec)	Corneal Power (mW)	Retinal Irradiance ($W.cm^{-2}$)	Radiant Exposure ($J.cm^{-2}$)
50	38.6 $\pm$ 0.7	16.4	819
100	34.3 $\pm$ 0.8	14.5	1450
500	30.5 $\pm$ 1.0	12.9	6548
1000	28.5 $\pm$ 0.7	12.1	12069

Parameters:

Seven monkeys
500 μm spot size
10 nm half bandwidth
Ocular media transmittance = .830

TABLE II

Corneal power, retinal irradiance and radiant exposure for cw radiation

860 nm (10 nm half bandwidth)

Exposure Time (sec)	Corneal Power (mW)	Retinal Irradiance ($W.cm^{-2}$)	Radiant Exposure ($J.cm^{-2}$)
10	44.3 $\pm$ 1.0	17.6	176
100	36.7 $\pm$ 1.4	14.6	1460
500	30.0 $\pm$ 1.4	11.9	5969
1000	27.7 $\pm$ 1.9	11.0	11000

Parameters:

Seven monkeys
500 μm spot size
10 nm half bandwidth
Ocular media transmittance = .780

TABLE III

Corneal power, retinal irradiance and radiant exposure for cw radiation

910 nm (55 nm half bandwidth)

Exposure Time (sec)	Corneal Power (mW)	Retinal Irradiance ($W.cm^{-2}$)	Radiant Exposure ($J.cm^{-2}$)
300	43.5 ± 1.5	15.9	4781
600	35.5 ± 1.5	13.3	6650
1000	30.0 ± 2.0	11.3	11250

Parameters:

Three monkeys
500 μm spot size
55 nm half bandwidth
Ocular media transmittance = .735

TABLE IV

Sensitivity of the rhesus retina to photic damage as function of wavelength for three aphakic eyes

Waveband 10 nm	Integrated Transmittance of Ocular Media T	100 s Exposure P_c μW	E $W.cm^{-2}$	H $J.cm^{-2}$	1000 s Exposure P_c μW	E $W.cm^{-2}$	H $J.cm^{-2}$
		420			60.1		
		433			55.0		
		365			71.0		
405	0.724	406±21	0.15	15.0	62.0±4.7	.023	23
		286			30.4		
		234			20.1		
		213			33.1		
380	0.652	244±22	.081	8.1	27.9±4.0	.0093	9.3
		153			21.9		
		204			22.0		
		233			16.4		
350	0.537	197±23	.054	5.4	20.1±1.9	.0055	5.5
		255			25.5		
		209			25.0		
		237			20.0		
325	0.422	234±23	.05	5.0	23.5±1.8	.0051	5.1

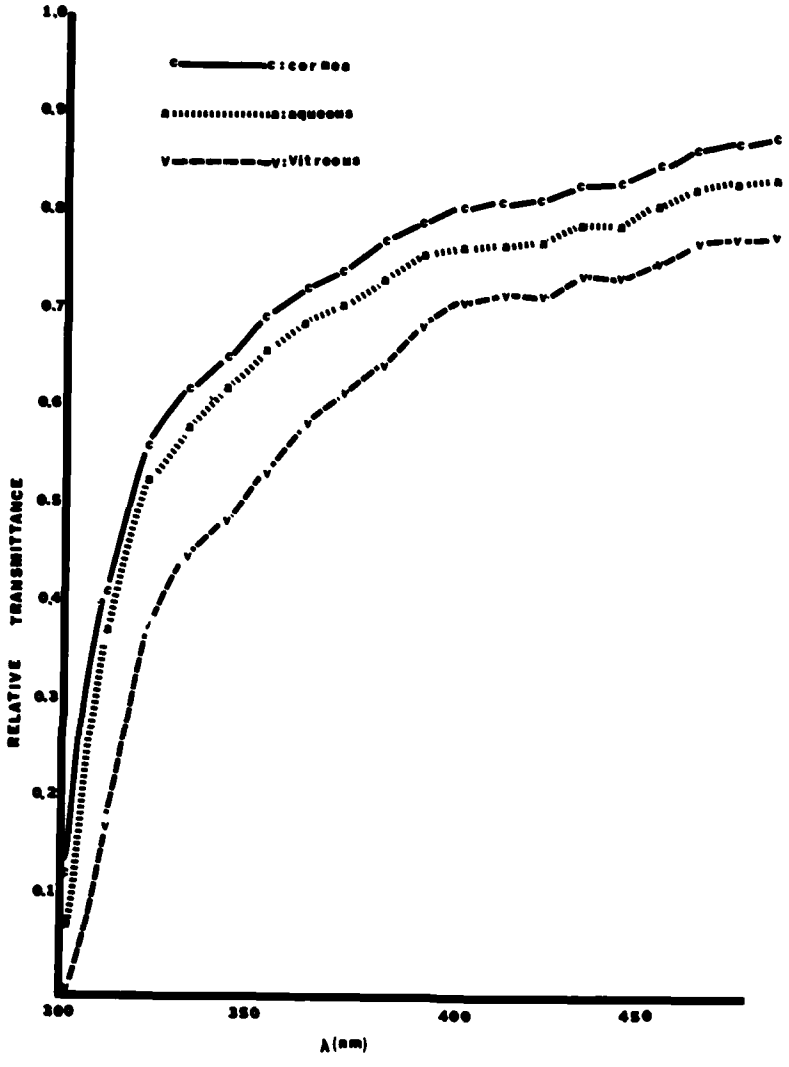
c : cornea
a : aqueous
v : vitreous
RELATIVE TRANSMITTANCE
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
300
350
400
450
λ(nm)

Figure 1

and the a-curve represents absorption in the aqueous. In like manner, the area between the a-curve and the v-curve denotes absorption in the vitreous. The v-curve gives the fraction of energy entering the eye which is incident on the retina for an aphakic rhesus monkey. The integrated transmittance for each of the four 10 nm wavebands was calculated from the v-curve.

The results of these exposures are summarized in Table IV where Column 1 gives the peak wavelength of each 10 nm bandpass, Column 2 gives the integrated transmittance through the ocular media of the aphakic eye for each wavelength using Maher's data for the rhesus monkey, Column 3 gives the power measured at the cornea in μW for 100 s exposures and Column 4 repeats Column 3 except that the exposures are 1000 s in duration. The measured power P_c is given for each aphakic eye, together with the average and standard deviation. From the average power P_c the retinal irradiance E in $W.cm^{-2}$ and retinal radiant exposure H in $J.cm^{-2}$ are caluclated for a 500 μm spot size using the transmittances given in Column 2.

The radiant exposures required to produce a minimal lesion range from 15 $J.cm^{-2}$ at 405 nm to 5 $J.cm^{-2}$ at 325 nm for 100 s exposures and from 23 $J.cm^{-2}$ to 5.1 $J.cm^{-2}$ for 1000 s exposures. There is no significant difference between the radiant exposures for the wavelengths 350 and 325 nm. Only approximately 20 μW of power entering the eye can produce a minimal retinal lesion in the aphakic rhesus eye in 1000 s (16.7 minutes). When one considers that 5 - 6 $mW.cm^{-2}$ of near UV radiation are present in sunlight at sea level on a clear summer day and that a 3 mm pupil would admit about 350 μW into the eye during direct sungazing it becomes apparent that the aphakic eye is extremely vulnerable to sunlight and near UV radiation in general. A minimal blue light retinal lesion (441 nm) requires a radiant exposure of about 30 $J.cm^{-2}$ (2) as compared to about 5 $J.cm^{-2}$ for 350-325 nm radiation. Thus, the rhesus retina is about 6 times more sensitive to near UV radiation as compared to blue light.

In Figure 2 we have plotted the action spectrum for near UV radiation using the irradiance needed on the retina to produce a minimal lesion in 100 s and in 1000 s as the biological endpoint. The reciprocal of irradiance in $W.cm^{-2}$ is plotted logarithmically along the ordinate vs wavelength in nanometers on the abscissa. For comparison we have plotted on the same graph the action spectrum for visible and near infrared radiation taken from our previous work (2) which gives the action spectrum for 8 laser lines. The small displacement between the

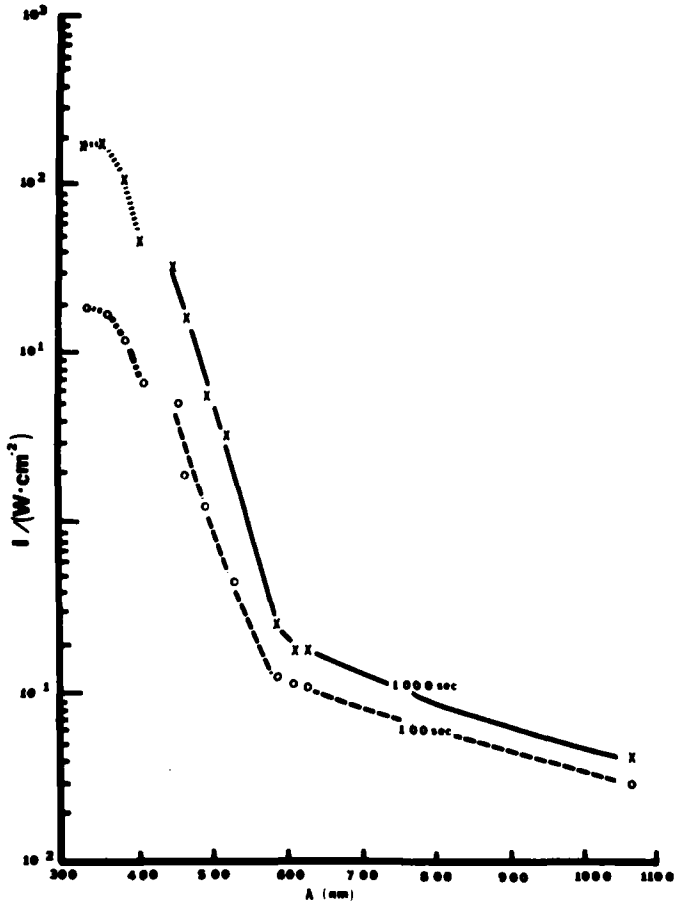

Figure 2

near UV action spectra and the visible action spectra is probably due to the difference in energy distribution on the retina since the xenon lamp optical system produces a flat irradiance across the retinal image, whereas the laser sources produce a Gaussian distribution. The former was 500 μm in diameter; the laser images were 500 μm to the $1/e^2$ points of the Gaussian distribution. From Figure 2 it can be seen that retinal sensitivity to photic damage continues to increase at least up to 350-325 nm in the near UV.

Funduscopic observation indicates that the minimal UV lesion differs from a blue light lesion in at least two respects. The lesion is funduscopically visible immediately after exposure and the size of the lesion is much smaller than the irradiated area. Both of these observations run contrary to our previous experience with short wavelength visible light where the lesion takes about 48 hours to appear and covers the entire irradiated area. To date we have histological data on one aphakic eye which was exposed to 350 nm radiation, spot size 500 μm, exposure time 100 s. Multiple exposures at different times before sacrifice provided minimal lesions at postexposure times ranging from 1 hour to 30 days. This eye was enucleated and prepared for LM and EM analysis by the methods previously described (3).

Figure 3 is a phase contrast unstained micrograph (80 X) of a minimal UV lesion at two days postexposure. The lesion which is about 100 μm across shows a marked thinning of the outer nuclear layer (ONL), loss of outer segments in both rods and cones and depigmentation of the retinal pigment epithelium (RPE) with clumping of melanin granules. There is severe damage to the photoreceptor cells which is in marked contrast to our observations on the blue light lesion where photoreceptor damage is minimal at two days after exposure.

Figure 4 (80 X),also unstained phase contrast, shows a 5 day lesion where a small group of photoreceptors are totally wiped out, including the ONL. Immediately below this wiped out area is a large clump of material containing melanin granules and other debris. This may be a large macrophage. To the left of this area the RPE is depigmented but the photoreceptors do not appear to be badly damaged. Why should a small group of photoreceptors be totally destroyed while the rest of the lesion shows only mild depigmentation? We have no answer to this enigma. We believe that the irradiance was fairly uniform across the irradiated area so that it would be unusual to find "hotspots" in the radiation beam. What is clear is that 350 nm radiation can be catastrophic to both rod and cone photoreceptors.

The next micrograph, Figure 5, is a higher magnification (125 X) of a ten day old lesion, unstained in phase contrast. While there is still some damage, especially in the outer segments of the photoreceptors, the main area of injury seems to be in the RPE which shows proliferation and depigmentation. There are a number of macrophages loaded with melanin granules in the subretinal space. This looks more like what we see at 10 days postexposure in the minimal blue light lesion. Perhaps by 10 days postexposure the uninjured photoreceptors have closed in and covered the small holes where rods and cones were obliterated by the UV radiation.

The last LM, Figure 6, is a low magnification (50 X) unstained phase contrast micrograph showing a 30 day UV lesion. No apparent damage can be seen in the photoreceptors and the RPE while somewhat depigmented looks otherwise normal. Again, this is more like the minimal blue light lesion a month after exposure. Presumably the photoreceptors while fewer in number have closed in to fill the gaps seen at 2 and 5 days postexposure. However, it should be emphasized that these were very mild or minimal lesions. Exposure to higher levels of near UV radiation could wipe out large numbers of rods and cones resulting in catastrophic and irreparable damage to the retina. We conclude that the primate retina is very vulnerable to near UV radiation. Since there are more than 400,000 cataract operations a year in the United States alone, there is a large population of people who are vulnerable to near UV radiation. Moreover, ordinary glass, Crown glass and most plastics readily transmit the near UV.

It is interesting to note that human rhodopsin and the cone pigments have high absorption peaks in the near UV. Rhodopsin has a peak around 350 nm which is about one-third that in the visible, while "green" cones have a near UV peak comparable to the visible peak. The blue cone has a high UV peak and only a shoulder in the visible (4). These absorption peaks of the photopigments in the near UV may help to explain why the primate retina is so sensitive to photic damage from the UVA.

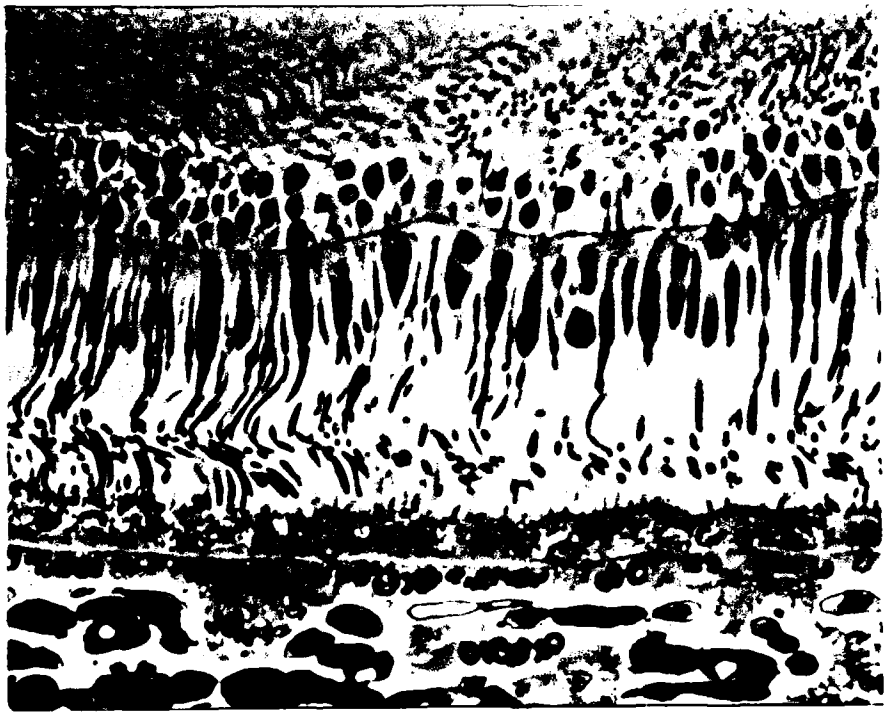

FIGURE 3. A phase contrast unstained light micrograph (80 X) of a minimal UV lesion at two days postexposure.

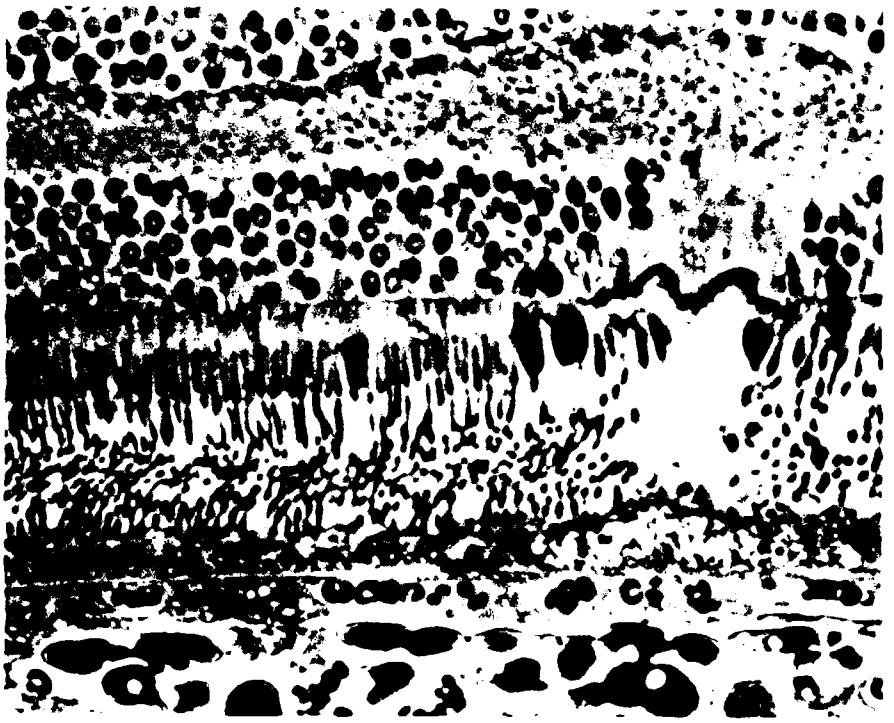

FIGURE 4. A phase contrast unstained light micrograph (80 X) of a minimal UV lesion at five days postexposure.

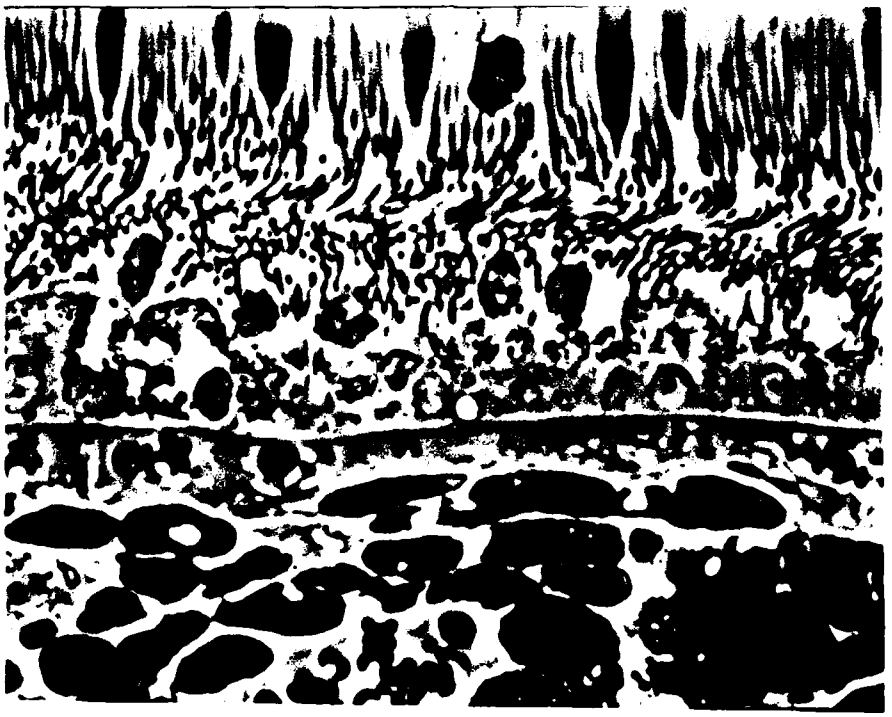

FIGURE 5. A phase contrast unstained light micrograph (125 X) of a minimal UV lesion at ten days postexposure.

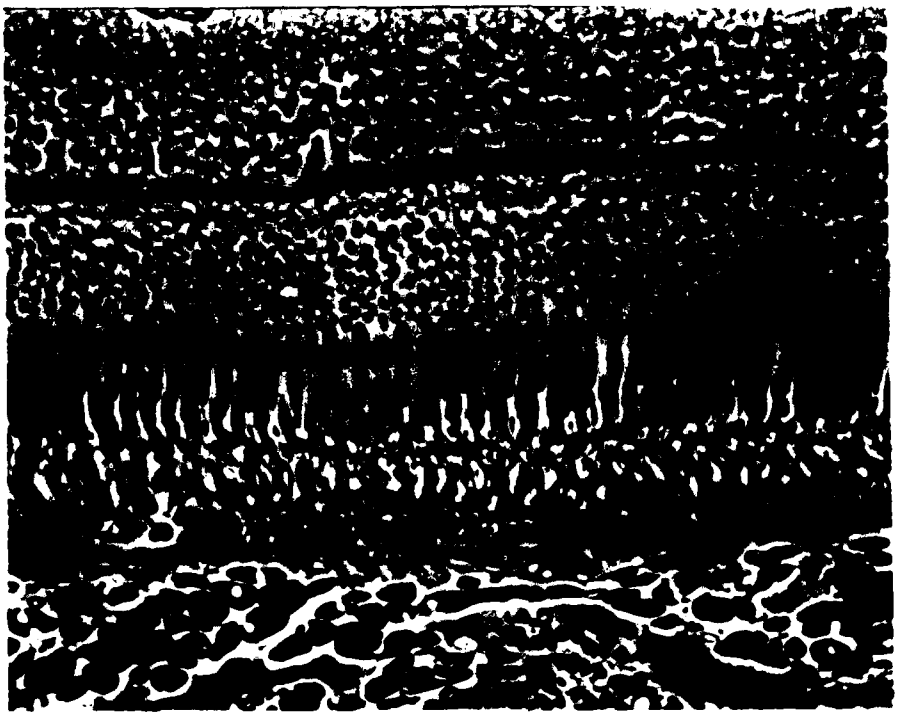

FIGURE 6. A phase contrast unstained light micrograph (50 X) of a minimal UV lesion at thirty days postexposure.

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4. Exposure of Trained Animals to Near UV and Blue Radiation

The purpose of this program is to determine whether intermittent, long term chronic exposure to radiation at levels consonant with the environment accelerates aging effects in the lens and retina, specifically senile cataract and senile macular degeneration.

Two rhesus monkeys have been trained for this program. The first animal started daily exposures to a near UV plus blue spectrum (330-490 nm) on Oct. 10, 1979. This animal had received 399 exposures as of Friday, June 5, 1981, when it died suddenly, presumably due to bloat on Friday night. When last examined by Dr. Kennon Guerry the retinal reflexes in the exposed eye appeared normal, but at the level of the pigment epithelium there was a diffuse granularity and more of a beaten metal appearance with numerous small round areas of pigment condensation located primarily on the nasal side of the macula. The unexposed or control eye had normal retinal reflexes and the pigment epithelium had a healthy and normal appearance. There was no distinguishable difference between the lenses in the exposed and control eye. Both lenses were clear and normal in appearance.

This animal was exposed to 5 $mW.cm^{-2}$ (330-490 nm) for 1000 s on a daily basis, 5 days per week. Approximately 27% of the energy between 400 and 490 nm reached the retina. During the first 120 exposures the retinal daily radiant exposure was 49 $J.cm^{-2}$. The radiation angle of divergence was increased from 2^{o} to 5.3^{o} to provide a larger image size on the retina. The daily radiant exposure for the next 279 exposures was reduced from 49 to 8 $J.cm^{-2}$. The accumulated radiant exposure to the retina at the time of death was 8069 $J.cm^{-2}$. The calculated radiant exposure to the anterior surface of the lens for a 1000 s daily exposure was 3.9 $J.cm^{-2}$. The accumulated radiant exposure for 399 days was 1556 $J.cm^{-2}$.

The loss of this animal was a severe blow to our program, especially since we were unable to prepare the eyes for LM and EM because death occurred over the weekend. The funduscopic appearance of the retina after long term intermittent exposures to blue light suggested that subtle changes were underway which might be interpreted as a type of macular degeneration similar to what we have seen in very mild blue light lesions at 60 to 90 days postexposure. No conclusions can be drawn regarding lens changes since we were unable to measure "in vivo" fluorescence or transmissivity in the control and exposed lenses because of technical difficulties. Slit lamp observation did not detect any differences between the control and exposed lenses, but this is not surprising when one considers the long

latency period before the appearance of radiation opacities. We are training another animal and hope to start exposures in the near future.

The other trained animal began exposures to a near UV spectrum (330-420 nm) on Feb. 20, 1980. The amount of this radiation beam reaching the retina is negligible because of absorption in the ocular media. This experiment was designed specifically to test the hypothesis that small daily exposures to near UV radiation produce aging effects in the lens leading eventually to senile cataract. Irradiance at the surface of the cornea is 5 $mW.cm^{-2}$ for 1000 s on a daily basis, 5 days per week. The daily radiant exposure at the surface of the lens is 3.6 $J.cm^{-2}$ and this animals has received 319 exposures as of June 5, 1981. Thus, the accumulated radiant exposure is 1148 $J.cm^{-2}$. When last examined by Dr. Kennon Guerry both exposed and control lenses appeared normal, as did the pigment epithelium in both eyes. We are hoping to perfect our techniques for measuring lens fluorescence and transmissivity so that we can apply more subtle criteria for lens changes.

Some progress along these lines has been made. Fluorescence measurements are underway with an optical system consisting of an integrating sphere (coated on the inside with Eastman reflecting paint), a quartz light guide and a photovoltaic detector with a 10 nm interference filter at 440 nm. The collimated exciting beam (330-420 nm) passes through a small port in the sphere and emerges through another small port on the opposite side into the quartz light guide which serves the double purpose of propagating the exciting beam to the eye and collecting the fluorescent light emitted by the lens. The uncollimated fluorescent light is reflected uniformly over the inner surface of the sphere. An EG&G UV enhanced photovoltaic cell, model 444B, is located at a third port in the sphere at 90^{o} to the entrance-exit ports. The 10 nm interference filter insures that only a narrow bandpass peaked at 440 nm can reach the detector.

The photovoltaic cell is connected to the inverting input of a chopper stabilized operational amplifier with a gain of 5×10^{9}. In this configuration the cell current feeds into a virtual ground, thus providing an effective short circuit for maximum linearity over 6 decades. A range of sensitivities is provided by switching in various values of feedback resistors from 1×10^{4} to 1×10^{9} Ohms. Preliminary results with unexposed cynomolgus monkeys have shown remarkable reproducibility.

Attempting to measure lens transmissivity in an anesthetized monkey presents technical difficulties which have not been wholly solved as of this writing. We are introducing the laser beam into the eye through a Goldman lens and attempting to measure the reflected beams from the anterior and posterior lens surfaces with the photovoltaic cell described above. We have been unable so far to separate the reflected beams sufficiently to measure them accurately. We are experimenting with a scanning technique which may solve this problem. Our major difficulty is reproducibility due to repetitive positioning of the exciting beam.

5. Long Term Repetitive Exposure of Aphakic Monkey to Near UV

The purpose here is to study the effects on the retina of an aphakic eye of long term repetitive exposure to a near UV spectral band 330-420 nm, roughly similar to a clear sunny environment.

Since the first of the year 6 animals have been rendered aphakic by operation in one eye. One animal died of bloat, 3 animals were used for data on retinal sensitivity to near UV radiation and histology was performed on one of these three. The fifth animal had vitreous clouding for the first two months after the operation. This animal now has a clear vitreous and is undergoing training for daily exposures to the spectral band 330-420 nm. The sixth animal is being used for additional LM and EM analysis of the UV retinal lesion.

The results on retinal sensitivity to near UV given in section 3 indicate that the corneal irradiation must be extremely low to avoid exceeding the threshold for minimal damage. For example, calculations show that a corneal irradiance of 170 $\mu W.cm^{-2}$ for 1000 s, assuming a pupillary diameter of 3 mm and a retinal spot size of 1 mm diameter would produce a radiant exposure of 1 $J.cm^{-2}$ to the retina. Thus, 5 exposures per week would equal the minimal lesion threshold of 5 $J.cm^{-2}$ if the daily radiant exposures were 100% cumulative. These calculations also assume that the monkey's eye would remain fixed during the 1000 s exposures, an assumption which is obviously false since the trained animal is constantly moving his eyes while scanning the screen and only fixes on the target when the Landolt ring appears. In actuality the 1 mm spot size on the retina is moving most of the time so that the entire macular area is being exposed. Maximum exposure should be centered on the foveola, however. This type of exposure provides an approximate simulation to that expected for an aphakic eye outdoors on a clear sunny day. The animal will be anesthetized for funduscopic examination at intervals of two weeks to a month as experience dictates.

6. Modulated vs CW Light on Monkey Retina

For reasons unknown the threshold for retinal damage is lower for repetitive pulse laser radiation than for cw laser radiation. A case in point are the findings for modulated GaAs radiation (830 nm) listed under section 2b, though a possible explanation is that the GaAs diode goes into high frequency oscillation producing picosecond pulses. Stuck, Lund and Beatrice (1) have made an exhaustive study of the available literature on pulsed vs cw laser radiation, and in every case the threshold for retinal damage is lower for repetitive pulses or pulse trains than for cw radiation. They have proposed two methods for the calculation of permisssible ocular exposure to repetitive pulse laser radiation.

The purpose of our program is to investigate further the above phenomenon utilizing a recently acquired krypton-argon acoustically modulated laser which can provide pulse widths and repetition rates over a wide range of values and at wavelengths from the red end to the blue end of the spectrum. We propose to study retinal sensitivity to damage as a function of wavelength, pulse repetition frequency and pulse duration in the train.

We have obtained data on two rhesus eyes using the highest modulation frequency available with our acoustic system (20.4 MHz, 49 ns pulses) at three wavelengths, 514.5, 488 and 458 nm. We have adopted the same experimental parameters used in our original program with 8 cw laser lines (2,3) so that these data can be compared to our earlier cw data. These parameters are as follows: 500 μm spot size to the $1/e^2$ points of the Gaussian distribution, exposure times of 1, 10, 100 and 1000 s, funduscopic examination at 48 hours postexposure as biological endpoint, and E_o in $W.cm^{-2}$ as calculated by the formula $E_o = P_c T/2\pi\delta^2$ where P_c is the power entering the cornea, T is the transmittance through the ocular media and δ is the Gaussian paramter in $E = E_o \exp(-r^2/2\delta^2)$.

These data are shown in Table V. Column 1 gives the wavelength in nm (parenthesis beside wavelength indicates monkey number) and 20.4 MHz modulation is compared with previous cw data at each wavelength as taken from Table 1 in reference (3). Column 2 gives the transmittance through the ocular media while the remaining columns give P_c in mW, H_o the retinal radiant exposure in $J.cm^{-2}$, E_o the retinal irradiance in $W.cm^{-2}$ and K the maximum retinal temperature in ^oC for exposure times of 1, 10, 100 and 1000 s.

TABLE V

The corneal power P_c, the retinal radiant exposure H_o, the retinal irradiance E_o, and the maximum retinal temperature K required to produce in 2 rhesus eyes a minimal lesion in 1, 10, 100 and 1000 s as detected funduscopically 48 hours postexposure are given for three wavelengths 514, 488 and 458 nm of laser light acoustically modulated at 20.4 MHz. These data (the average for two eyes) are compared to similar data for cw laser light as taken from Table I in Reference (3). The 16 s exposures are extrapolated back to 10 s by means of Figure 1 in Reference (3).

		1 s Exposure				10 s Exposure				100 s Exposure				1000 s Exposure			
Wavelength in nm	Trans-mittance	P_c mW	H_o J.cm^{-2}	E_o W.cm^{-2}	K ^oC	P_c mW	H_o J.cm^{-2}	E_o W.cm^{-2}	K ^oC	P_c mW	H_o J.cm^{-2}	E_o W.cm^{-2}	K ^oC	P_c mW	H_o J.cm^{-2}	E_o W.cm^{-2}	K ^oC
514 (475)*	.87	21.8	19.3	19.3	27	16.5	150	15	20	5.35	470	4.7	8	.25	220	.22	0.8
514 (803)*	.87	16.5	14.6	14.6	25	12.8	110	11	18	4.8	430	4.3	7	.308	270	.27	0.9
514 20.4MHz	.87	19.2	16.7	16.7	26	14.7	130	13	19	5.1	450	4.5	7.5	.28	245	.25	0.85
514 cw	.87	16.3	14.5	14.5	25	11.6	100	10	17	2.5	220	2.2	4	.36	320	.32	1.0
488 (475)*	.83	18.4	15.5	15.5	20	8.4	71	7.1	10	1.93	160	1.6	2	.165	140	.14	0.1
488 (803)*	.83	12.3	10.4	10.4	18	9.0	76	7.6	11	1.70	144	1.4	2	.172	150	.15	0.2
488 20.4MHz	.83	15.4	13.0	13.0	19	8.7	74	7.4	10.5	1.8	152	1.5	2	.169	145	.145	0.15
488 cw	.83	11.0	9.4	9.4	17	8.3	70	7.0	10	0.9	77	0.77	1	.17	150	.15	0.1
458 (475)*	.69	8.5	6.0	6.0	9	7.4	52	5.2	8	.74	52	0.52	1	.09	63	.063	0.1
458 (803)*	.69	8.6	6.0	6.0	9	7.7	54	5.4	8	–	–	–	–	.11	77	.077	0.2
458 20.4MHz	.69	8.55	6.0	6.0	9	7.55	53	5.3	8	.74	52	0.52	1	.10	70	.070	0.15
458 cw	.69	7.3	5.1	5.1	10	7.1	50	5.0	7	.74	52	0.52	1	.08	60	.06	0.1

*Denotes the monkey number. Two eyes in two different animals were exposed.

With one exception (1000 s exposure to 514 nm) the cw thresholds are lower than the 20.4 MHz thresholds, though probably not significantly lower. The 20.4 MHz thresholds are the average for only two eyes in two different animals, whereas the cw thresholds represent the average of 9 eyes, each in a different animal. Therefore, it would be unwise to draw any conclusions until more data are available. We plan to place minimal lesions from both cw and 20.4 MHz laser radiation side by side in the same eye for comparison and histological analysis. We also plan to extend this study to the 647 nm wavelength from the krypton-argon laser.

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1. Stuck, B.E., Lund, D.J. and Beatrice, E.S. Repetitive pulse laser data and permissible exposure limits. Institute Report No. 58. Div. of Non-Ionizing Rad., Dept. Biomedical Stress, Letterman Army Institute of Research, Presidio of San Francisco, CA 94129 (April 1978).
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7. Publications: 1980-1981

a. Ham, W.T.Jr., Ruffolo, J.J.Jr., Mueller, H.A. and Guerry, D. III. The nature of retinal radiation damage: dependence on wavelength, power level and exposure time. Vision Res. 20, 1105-1111 (1980).
b. _____, Mueller, H.A. and Ruffolo, J.J.Jr. Retinal effects of blue light exposure. SPIE Vol. 229, Ocular Effects of Non-ionizing Radiation (1980). Society of Photo-Optical Instrumentation Engineers, Box 10, Bellingham, WA 98227.
c. Clarke, A.M. Blue light exposure and long term deficits in visual function. SPIE Vol. 229, Ocular Effects of Non-ionizing Radiation (1980). Society of Photo-Optical Instrumentation Engineers, Box 10, Bellingham, WA 98227.

Abstracts: 1980-1981

d. Ham, W.T.Jr., Mueller, H.A. and Ruffolo, J.J.Jr. Near UV action spectrum for retinal damage. ARVO 1981, Suppl. of Invest. Ophthalmol. and Vis. Sci. pp. 164 (1981).

e. Ruffolo, J.J.Jr., Ham, W.T.Jr. and Mueller, H.A. Retinal photopathology: distinction between photochemical and thermal lesions. ARVO 1981, Suppl. of Invest. Ophthalmol. and Vis. Sci. pp 162 (1981).

f. Ham, W.T.Jr., Mueller, H.A., Ruffolo, J.J.Jr., Guerry, D.III and Guerry, R.K. Retinal sensitivity of rhesus monkey to near UV radiation. 9th Annual Meeting, Am. Soc. for Photobiology, Program and Abstracts, pp 180, June 14-18, 1981.

8. Additional Activities: 1980-1981

Dr. Ham reviewed papers and research proposals for the following during 1980-1981: National Science Foundation, National Eye Institute (NIH), Investigative Ophthalmology & Visual Science (2 papers), Health Physics Journal, book review for American Journal Ophthalmology, Bioelectromagnetics, and Applied Optics.

August 26-27, 1980: Dr. Ham attended a two day meeting of the Electromagnetic Research Management Advisory Council (ERMAC) of which he is a charter member.

November 10, 1980: Drs. Ham and Kennon Guerry visited Bell Labs, Murray Hill, N.J. and gave invited lectures on ocular hazards associated with near infrared radiation (800-1600 nm) and the medical and legal aspects of a protocol for eye examinations.

December 16, 1980: Dr. Ham gave an invited paper on "Ocular damage from near ultraviolet and blue portion of the spectrum" before the annual meeting of the American Society of Optometry at the Drake Hotel, Chicago.

April 26 - May 1, 1981: Drs. Ham and Ruffolo and Mr. Mueller attended the annual meeting of ARVO at Sarasota, Florida and gave two papers, abstracts of which are enclosed.

June 14-18, 1981: Dr. Ham and Mr. Mueller attended the annual meeting of the American Society for Photobiology at Williamsburg, VA and gave a paper, the abstract of which is enclosed.

July 15-16, 1981: Dr. Ham attended another two day meeting of the Electromagnetic Research Management Advisory Council (ERMAC) in Washington, D.C.